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REVIEW

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Building a secretory apparatus: role of ARF1/COPI in Golgi biogenesis and maintenance

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Abstract The secretory apparatus within all eukaryotic cells comprises a dynamic membrane system with bidirectional membrane transport pathways and overlapping compartmental boundaries. Membrane traffic and organelle biogenesis/maintenance are fundamentally linked within this system, with perturbations in membrane traffic quickly leading to changes in organelle structure and identity. Dissection of the molecular basis of these properties in yeast and mammalian cells has revealed a crucial role for the cytoplasmic protein complex ARF1/COPI, which undergoes regulated assembly and disassembly with membranes. ARF1/COPI appears to be involved in the formation and maintenance of the Golgi complex, which is the receiving and delivery station for all secretory traffic. ARF1-GTP, through assembly of COPI to membranes and, possibly, through activation of PLD, is likely to promote the formation and maturation of pre-Golgi intermediates into Golgi elements, whereas ARF-GDP causes COPI dissociation and stimulates the formation of retrograde transport structures that recycle Golgi membrane back to the ER. These processes are appear to underlie the coupling of organelle biogenesis and membrane trafficking within cells, allowing the size and shape of secretory organelles to be altered in response to changing cellular needs. Future work needs to address how the activation and localization of ARF1/COPI to membranes as well as other related factors are temporally and spatially regulated, and by what mechanism they transform membrane shape and dynamics to facilitate protein transport and compartmental functioning.

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Secretory trafficking viewed as a system

The goal of a ‘top-down’ approach, to secretory traffic is to determine general properties of secretory membrane traffic that are common to diverse cell types and thus are likely to be important for secretion. Present- day cells are the products of an unfolding of millions of years of fine-tuning - a great historical testing of niches - constrained only by molecular hardware and genetic coding. It is not surprising, therefore, that many aspects of secretory traffic, including the roles of cytoskeletal elements and the sizes, shape, and location of organelles, vary considerably in different cell types. Despite this, several features of secretory traffic are common to simple protozoa, yeast, and mammalian cell types and, therefore, are likely to be fundamental to all secretory trafficking systems. The following list summarizes many of these common properties:

1. The secretory pathway functions through the activities of membrane-bound structures composed of amphiphilic lipids with attached or inserted proteins.

The universal character of these structures derives from the ability of phospholipid molecules to assemble into sheet-like structures, producing a unit membrane with three phases: exterior, barrier, and lumen. Vectorial translocation of newly synthesized protein into the lumenal space of these membranes is the first step in the process of protein secretion. Because the lumen of these membranes is topologically equivalent to the cell exterior, proteins carried within this space will be released into the extracellular environment upon fusion of membrane transport intermediates with the plasma membrane. Regulated association of cytoplasmic proteins to the surface of membranes involved in secretory traffic can promote sorting and retention of specific protein and lipid species; it also can lead to their morphological transformation into flattened cisternae, tubules or vesicles. This enables membranes involved in secretion to differentiate and self-organize into functional domains and compartments. With compartmentalization of its secretory membranes, a cell can modify newly synthesized secretory

products in a series of controlled steps, store molecules until needed, and then deliver them to specific cell surface domains.

2. The diverse compartments comprising the secretory pathway are all derived from the endoplasmic reticulum (ER), which itself arises from the continuous insertion of newly synthesized lipid and protein into previously existing ER membrane.

The ER is the port of entry for all membrane and protein into the secretory pathway and engages in lipid biosynthesis and metabolism, as well as in protein synthesis, folding, assembly, and degradation (Sitia and Meldolesi 1993; Lippincott-Schwartz 1994). It is comprised of an extensive array of interconnecting membrane tubules and cisternae which usually extend throughout the cell (Terasaki et al. 1986). A variety of specific resident ER components participate in folding and processing of newly synthesized proteins (Hurtley and Helenius 1989). Folding and assembly of proteins is also facilitated by the specialized lumenal environment of the ER, which is oxidizing (promoting disulfide bond formation) and has high ATP and free calcium levels. Together, these properties of the ER enable newly synthesized lipid and protein to be converted from low energy, monomeric forms to higher energy, multimeric complexes.

3. Protein export from the ER occurs only when a protein is properly folded and assembled, and takes place in specialized regions of the ER adjacent to clusters of elaborate membrane tubules/vesicles.

Properly assembled protein complexes usually do not freely leave the ER system, but must be actively sorted into ER exit sites (Mizuno and Singer 1993; Balch et al. 1994; Barlowe et al. 1994), which are smooth regions of the ER devoid of ribosomes that are adjacent to elaborate tubule clusters (also called vesicle tubular clusters; Bannykh et al. 1996, pre-Golgi intermediates; Saraste and Svensson 1991; Saraste and Kuismanen 1992; Presley et al. 1997, or the intermediate compartment; Schweizer et al. 1990). ER exit sites are sometimes localized to discrete regions of the ER, as in the simple protozoan *Toxoplasma gondii*, where they localize to the apical region of the nuclear envelope (Sheffield and Melton 1968). In other cell types, they arise spontaneously throughout the ER (Presley et al. 1997). In all cases, they comprise relatively large domains (1–2 μm in diameter) that contain multiple budding profiles (and never single ER buds in isolation). Although budding of vesicles is widely believed to occur from ER exit sites (Palade 1975; Barlowe et al. 1994; Bannykh et al. 1996), ultrastructural studies have shown these sites sometimes consist of permanent/intermittent connections with the elaborate tubule clusters comprising pre-Golgi intermediates or with the first cisternae of the Golgi stack itself (see Clermont et al. 1994; Krijnse Locker et al. 1994; Stinchcome et al. 1995; Cole et al. 1996; Hermo and Smith 1998). This indicates, therefore, that the boundary separating the ER from the rest of the secretory pathway is not always distinct and that export of protein out of the ER does not necessarily rely on the production of small vesicles. It does, however, depend on energy. In the absence of ATP, or when energy-coupled regulatory molecules are inactivated/depleted, all protein export out of the ER ceases (Balch et al. 1986; Lippincott-Schwartz et al. 1990; Verde et al. 1995). The energy requirement for ER export could be for the morphological transformation of membranes occurring at ER exit sites (i.e., budding of membranes into tubular clusters), for the active recruitment and concentration of membrane and soluble cargo at these sites, or both. The concentration of protein cargo at ER exit sites and in pre-Golgi intermediates is likely to involve positive sorting signals to promote concentration (Nishimura and Balch 1997) and the selective removal of membrane and fluid once protein and lipid have entered these structures (Tang et al. 1995). The net result is a higher level of organization and ordering of protein and lipid within these membrane domains.

4. After their formation, pre-Golgi intermediates have only a transient existence before delivering their cargo uniquely to the Golgi complex in a process which couples forward secretory transport with Golgi biogenesis.

One view of the cargo-enriched tubular clusters adjacent to ER exit sites postulates that they are stable intermediates from which small vesicles bud to deliver cargo into the Golgi complex (Lotti et al. 1992). Recent time-lapse imaging of secretory trafficking using GFP chimeras, however, has demonstrated that this is not the case (Presley et al. 1997; Scales et al. 1997). Rather, such structures were found to serve as transport vehicles for delivery of protein and lipid through the cytoplasm to the Golgi complex after detaching and/or further differentiating from the ER. Pre-Golgi intermediates also have been shown to undergo maturation (by recycling of selected components back to the ER; Tang et al. 1995) and to be capable of homotypic fusion (Rowe et al. 1998). These findings suggest that pre-Golgi intermediates are the direct precursors of Golgi elements, with Golgi cisternae formed by continuous maturation/differentiation of pre-Golgi intermediates (Saraste and Kuismanen 1992; Lippincott-Schwartz 1993). Under this model, the *cis* face of the Golgi complex represents the site where pre-Golgi intermediates first merge together, while the *trans* face is where they have undergone further maturation through recycling pathways (Bannykh and Balch 1997; Glick et al. 1997; Mironov et al. 1997). This explains the existence of polarized entry and exit faces of the Golgi complex, since forward traffic is coupled to maturation. In some cells, maturation of pre-Golgi intermediates is coupled with the translocation of these structures along cytoskeletal elements to distinct regions of the cell (Presley et al. 1997; Scales et al. 1997) leading to Golgi membrane localization at these sites (Fig. 1).

5. The Golgi complex represents a dynamic steady-state system of membrane input and outflow and functions to process glycoproteins/glycolipids and to package them for delivery to the cell surface.

A potential mechanism for establishing the Golgi steady-state system involves a ‘fractionation’ of surface-destined membrane components from ER/Golgi resident components (Tang et al. 1995; Bannykh and Balch 1997;

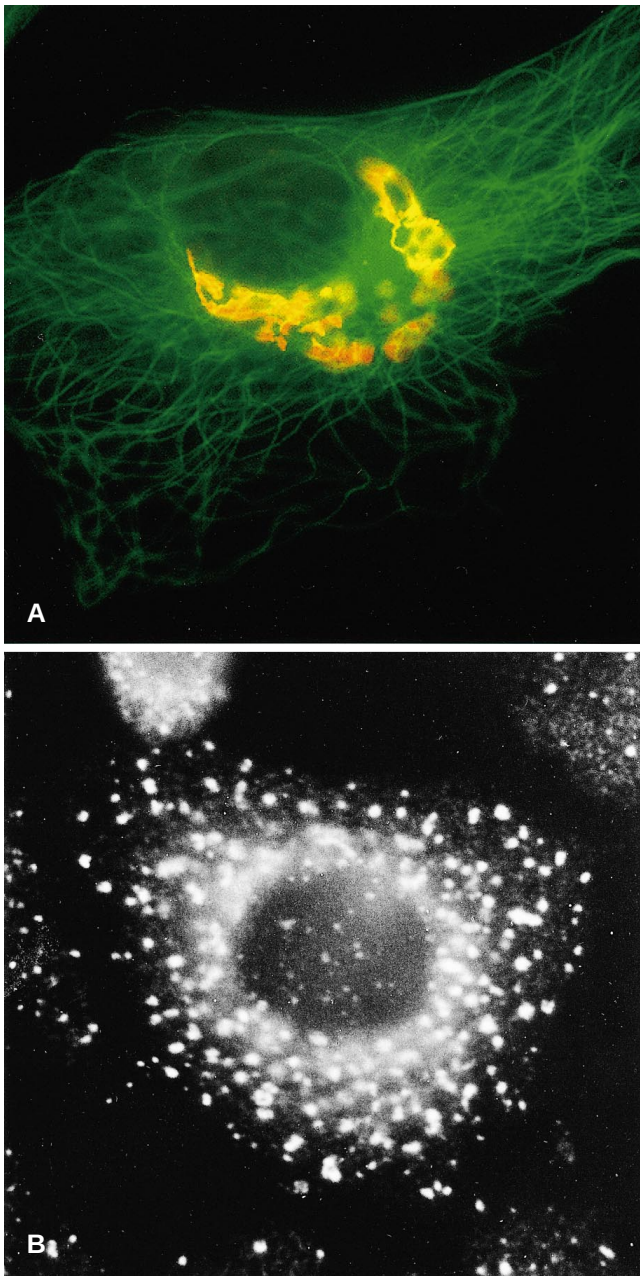

Fig. 1 The Golgi complex in mammalian cells (*orange antibody staining* in **A**) is usually localized adjacent to the nucleus where microtubules (green antibody staining in **A**) arise from the centrosome. This localization is dependent on continual input from pre-Golgi intermediates, which use microtubules to translocate into the Golgi region from peripheral endoplasmic reticulum (ER) exit sites (Presley et al. 1997; Scales et al. 1997). In the absence of microtubules, pre-Golgi intermediates are unable to translocate inward (Presley et al. 1997) and they accumulate recycling components (including Golgi enzymes; Cole et al. 1996). This results in Golgi stack reformation at peripheral ER exit sites scattered throughout the cell. The end result of this process is shown in **B**, which shows the distribution of Golgi membranes labeled with antibodies to the Golgi enzyme mannosidase II in cells treated with nocodazole for 3 h

Rowe et al. 1998). Fractionation begins at ER exit sites with concentration/sorting of newly synthesized surface-destined proteins (Balch et al. 1994), membrane trafficking machinery (Barlowe et al. 1994), and Golgi enzymes (Cole et al. 1996), and continues as pre-Golgi intermediates mature into Golgi elements. Membrane trafficking machinery and other molecules that do not partition efficiently into the sorting domains of these structures would recycle back to the ER; so would Golgi enzymes, but more slowly (Cole et al. 1998). The net result would be enrichment of Golgi enzymes and substrates in Golgi elements. This would generate an organizational complexity within Golgi membranes that allows elaborate carbohydrate processing reactions to be coordinated. The highly processed secretory products formed from these reactions could then enter, and possibly help differentiate, the *trans* Golgi network (Rambourg et al. 1979; Roth et al. 1985; Griffiths and Simons 1986; Ladinsky et al. 1994), which packages these products into transport intermediates for delivery to the cell surface.

For fractionation to result in Golgi biogenesis, molecules must be recycled back to the ER. This enables pre-Golgi and Golgi membranes to become selectively enriched in certain proteins and lipids. Many proteins have been shown to recycle to the ER, and at distinct rates. These include escaped ER resident components (Pelham 1991), membrane machinery necessary for ER-to-Golgi traffic (Sato et al. 1995; Zhang et al. 1997), and Golgi enzymes (Cole et al. 1996, 1998). The mechanism of retrograde trafficking is currently unclear, but is likely to be regulated (Pelham 1991; Hsu et al. 1992; Echard et al. 1998) and to involve lipid partitioning to segregate recycling components from forward moving cargo (Cole et al. 1998). The Golgi complex is known to be a major site of lipid transition within cells. Its lipid composition is intermediate between that of the ER (whose lipids and proteins tend to form thin bilayers) and the plasma membrane (whose lipids, including cholesterol, and proteins form thicker bilayers; Orci et al. 1981; Van Meer 1989; Coxey et al. 1993; Munro 1995). Changes in lipid composition across the Golgi complex, therefore, may establish a gradient of bilayer thickness, allowing highly mobile Golgi enzymes (Cole et al. 1996) and other recycling molecules to laterally partition into thinner membrane domains (Bretscher and Munro 1993). These thinner membrane domains might then be the source of recycling transport intermediates. The finding that increasing the length of the transmembrane domain of several Golgi and ER proteins results in their movement to the plasma membrane is consistent with this thinking (Munro 1995; Colley 1997; Yang et al. 1997; Cole et al. 1998).

The transport intermediates which recycle proteins back to the ER are believed to be either tubules or vesicles that pull off from Golgi rims (Pelham 1994; Mironov et al. 1997; Sciaky et al. 1997). Their simplicity contrasts with the complexity of pre-Golgi intermediates and Golgi elements that are engaged in forward movement within the secretory pathway. The latter undergo maturation and differentiate into elaborate flattened stacks of

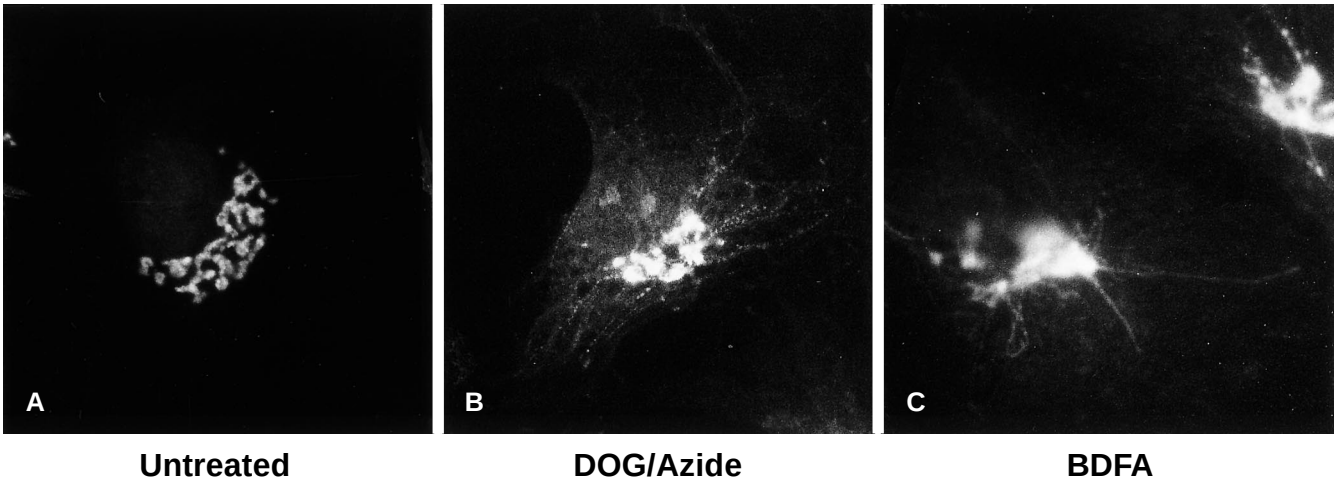

Fig. 2A–C Effect of energy depletion and brefeldin A (BFA) on the distribution of Golgi membranes in NRK cells. **A** Untreated cell. **B** Cell treated for 10 min with deoxyglucose and sodium azide to lower ATP levels. **C** Cell treated for 5 min with BFA. Cells were permeabilized and stained with antibodies to the Golgi enzyme mannosidase II followed by fluorescently labeled secondary antibodies. Note the extension of long tubules from the Golgi complex under conditions of either energy depletion or BFA treatment. Because both of these treatments leads to the dissociation of peripheral coat proteins from the Golgi complex (Donaldson et al. 1990), the data suggest that energy-coupled membrane association of cytosolic proteins is necessary for maintaining the integrity of the Golgi complex

cisternae in a process that is energy dependent. Under conditions of moderate energy depletion or when peripheral proteins are dissociated from Golgi membranes with brefeldin A (BFA), pre-Golgi and Golgi elements lose their organization and their membrane components enter retrograde tubule transport intermediates which can fuse with the ER (Donaldson et al. 1990; Lippincott-Schwartz et al. 1990; Cluett et al. 1993; Weidman et al. 1993; Sciaky et al. 1997) (see Fig. 2). This suggests that energy-coupled membrane association of peripheral proteins is necessary for forward distillation of secretory cargo within pre-Golgi and Golgi cisternae and for preventing such cargo from entering recycling transport intermediates.

Once the dynamic steady-state flux of proteins through forward and recycling pathways of the Golgi complex has become established, glycoprotein and glycolipid processing reactions become efficient, and give rise to highly processed and complex protein and lipid products. These products, in turn, are packaged into transport intermediates which bud off the *trans* face of the Golgi complex, translocate through the cytoplasm, and fuse with the plasma membrane.

Requirements for a minimal secretory system

The above list summarizes common features of secretory traffic that are shared by most cell types. Here we address the basic equipment necessary for secretion. A number of protozoan parasites have been used as model systems for ‘stripped down’ versions of eukaryotic cells. Some of these parasites (e.g., *Theilera*) represent the smallest known eukaryotic cell (measuring less that 1 μm in diameter; L. Tilney, personal communication). Despite their small size, many of these parasites contain a secretory apparatus with morphological properties that are nearly indistinguishable from those of higher eukaryotic cells. This is shown in Fig. 3 where the secretory apparatus of the primitive parasite *T. gondii* is shown diagramatically and by thin section. Note that despite its small size, the secretory membrane system of *Toxoplasma* is remarkably similar to that of higher eukaryotic cells. This indicates that secretory membranes have the ability to scale up or down their overall form to accommodate to different size environments.

Although *Toxoplasma gondii* contains an easily recognizable Golgi complex, many other simple organisms such as budding yeast and *Plasmodium* have less recognizable Golgi structures (Banting et al. 1995; Rambourg et al. 1995; Haldar 1996; Lingelbach 1997). Strikingly, *Theilera* and *Giardia lamblia* trophozoites possess no noticeable Golgi complex at all, even though they contain a membrane system that secretes and targets digestive enzymes. Results from studies of *G. lamblia* are particularly intriguing (Lujan et al. 1995). This parasite belongs to the earliest identifiable lineage among eukaryotic cells. Its ribosomal RNA shares more sequence homology with prokaryotes than with eukaryotes and it lacks mitochondria. Despite its primitiveness, *Giardia* engages in both constitutive and regulated protein secretion and contains the GTP-binding protein ARF (ADP-ribosylation factor) and the coat protein complex known as COPI (Rothman 1994; Lujan et al. 1995). Association of ARF and COPI with secretory membranes has been shown to be necessary for maintenance and proper functioning of the secretory pathway in higher eukaryotes and yeast (Klausner et al. 1992; Rothman and Wieland 1996; Gaynor and Emr 1997; Gaynor et al. 1998).

Trophozoite forms of *Giardia* colonize the small intestine of human hosts (giving rise to enteric disease worldwide). In the large intestine, induction of *Giardia* cyst wall proteins and their secretion transform tropho-

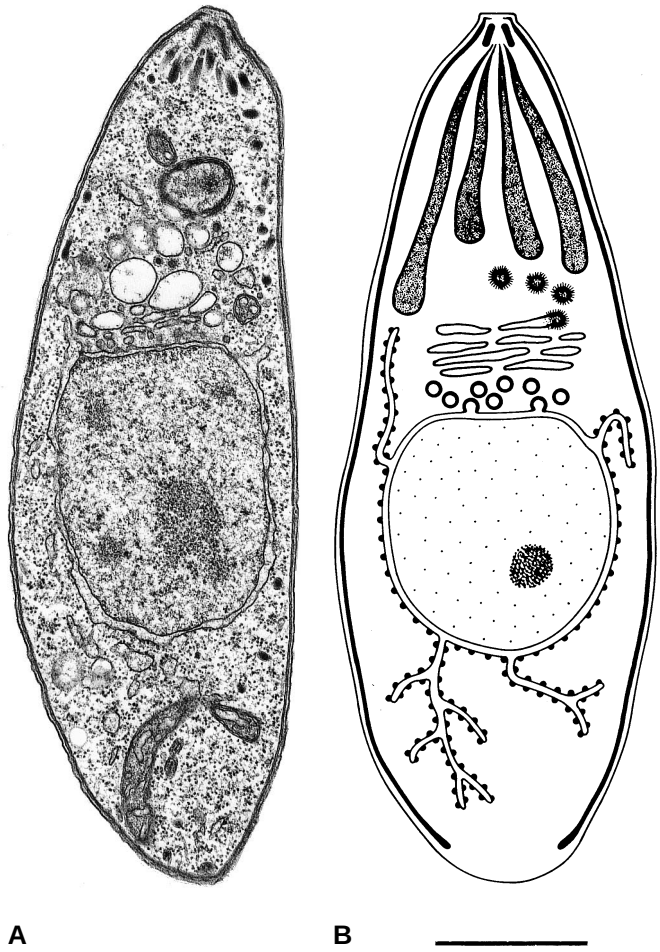

Fig. 3 Thin section of *Toxoplasma gondii*. (**A**) and diagram of its secretory membranes (**B**). *Bar* 1.0 μm. Note that although the overall size of *Toxoplasma* is extremely small, the organization of its secretory membranes is nearly identical to that of higher eukaryotes. This includes an ER that extends peripherally in the cytoplasm, a flattened stack of cisternae comprising the Golgi complex and transport intermediates. In *Toxoplasma*, vesicles appear to bud off the apical surface of the nuclear envelope and fuse to form the Golgi complex

zoites into cysts which are excreted in feces. Prior to encysting, *Giardia* trophozoites have no morphological or biochemically identified Golgi complex, yet can secrete simple non-glycoslyated proteins in a BFA-sensitive manner (Lujan et al. 1995). Within 4 h of placing non-encysting cells into encysting medium, however, induction of Golgi enzyme activities and the appearance of a Golgi structure occurs. Developmental signals, therefore, appear to coordinate the biosynthesis of Golgi enzymes and carbohydrate-containing secretory substrates for building a Golgi complex. This allows efficient cyst wall production in encysting trophozoites.

These results suggest that the minimal machinery for protein secretion in primitive eukaryotic cells like *Giardia* utilizes ARF and COPI but does not require a morphologically or biochemically identifiable Golgi complex to secrete simple non-glycosylated proteins. Golgi biogenesis in *Giardia* appears only with the induction of Golgi resident enzymes needed for synthesis of carbohydrate-rich cyst wall components during encystation. Thus, an important characteristic of the secretory apparatus is its ability to accommodate a wide variation in quantity and character of molecules being exported from the ER. The coupling of membrane traffic with organelle biogenesis appears to be an ideal mechanism for achieving this flexibility.

Molecular components of secretory trafficking

The basic eukaryotic secretory apparatus appears to consist of transport machinery that has evolved to perform three fundamental tasks: (1) sorting and concentration of secretory products for exit out of the ER; (2) differentiation of membrane-bound transport intermediates (i.e., organellogenesis); and (3) regulated fusion of transport intermediates with target membranes. Insight into the molecular machinery that accomplishes these tasks has come from genetic and biochemical approaches. In *Saccharomyces cerevisiae*, for example, more than 15 genes have been identified that are required for membrane traffic between the ER and Golgi complex (see Schekman and Orci 1996). Mammalian homologues for many of these genes have been identified biochemically from cell-free systems (Beckers et al. 1989; Clary et al. 1990; McNew et al. 1997), and through cloning based on sequence similarity with known yeast genes. At the cellular level, transport between different organelles appears to occur by universally conserved mechanisms. Compartmental specificity is provided by distinct members of protein families, such as those involved in sorting (e.g., membrane coats, adaptor proteins, and cargo receptors, see Kirchhausen et al. 1997; Kuehn and Schekman 1997) and membrane fusion (Rabs, SNARES, see Hay and Scheller 1997; Novick and Zerial 1997; Rothman and Sollner 1997). The conservation of this machinery appears to extend throughout the Eukarya (Satiat-Jeunemaitre et al. 1996; Van Wye et al. 1996), and demonstrates a fundamental role for these molecules in even the simplest organisms.

Studies in yeast and mammalian cells have demonstrated that transport from the ER to the Golgi complex, as well as in maintenance of Golgi structure, requires the activities of small ras-related GTPases whose primary role is to initiate the assembly of distinct cytosolic coat protein complexes on ER-derived membranes. These complexes, termed COPII and COPI, function primarily to concentrate cargo for ER export (see Bannykh and Balch 1998) and to maintain selected molecules within the ER/Golgi system (Letourneur et al. 1994; Dominguez et al. 1998), respectively.

COPII assembly originates with the Sec12p-mediated nucleotide exchange of GTP for GDP on the small GTPase Sar1p (reviewed in Schekman and Orci 1996). What triggers this exchange is currently unknown, but is likely to involve cargo molecules themselves or putative cargo receptors. Sec13/31p and Sec23/24p complexes are then

assembled onto the cytoplasmic leaflet of the activated ER membrane to form a COPII-coated bud. This is thought then to give rise to a COPII-coated vesicle (Barlowe et al. 1994; Bednarek et al. 1995; Bannykh et al. 1996). If the bud were not severed, however, a tubule might arise (Klausner et al. 1992) or a cluster of tubules if COPII assembled over a large surface area (see Fig. 4). That the COPII coat serves to concentrate cargo within the budding structure and is essential for protein exit out of the ER has been demonstrated both in yeast (Kuehn et al. 1998) and mammalian cells (Balch et al. 1994). Temperature-sensitive Sec23p (the GTPase activating protein, GAP, for Sarlp) mutants in yeast show a complete lack of protein exit from the ER (Yoshihisa et al. 1993; Gaynor and Emr 1997) and dominant-negative forms of Sar1p, as well as anti-Sar1p antibodies, prevent exit of cargo (i.e., VSVG) from the ER in mammalian cells (Kuge et al. 1994). The mechanism by which cargo becomes concentrated within this ER export structure is not clear. However, the cytoplasmic domains of numerous transmembrane proteins, several of which are putative cargo receptors, have been shown to bind sec23p in vitro (Kappeler et al. 1997; Dominguez et al. 1998).

While COPII functions to sort cargo solely within the ER membrane, assembly of the COPI coat is necessary for the subsequent differentiation of post-ER intermediates and early Golgi compartments. The mechanism by which COPII membranes 'hand-off' membrane-bound cargo to form COPI-coated transport intermediates is unclear, but is thought to involve a sequential coupling of COPII uncoating with COPI binding (Aridor et al. 1995; Rowe et al. 1996). The COPI coat is composed of seven subunits of a cytosolic protein complex called coatomer (Rothman 1994). The COPI coat has been localized to pre-Golgi intermediates, along the *cis* face of the Golgi and associated with the rims of subsequent cisternae (Oprins et al. 1993; Griffiths et al. 1995). Assembly of COPI onto membranes requires activation of the small GTPase, ARF1 (Donaldson et al. 1992a; Palmer et al. 1993). There are five human ARF proteins. ARF1 and 3 (96% identical) are the most abundant (Cavenagh et al. 1996). They have been localized to the Golgi complex and shown to recruit COPI there (Peters et al. 1995; Liang and Kornfeld 1997). The functions of other ARFs are not clear. Most studies of ARF's role in the Golgi complex have utilized ARF1 and there is no reason to suspect that the activity of ARF3 is substantially different.

A wealth of data, both in yeast and in mammalian cells, has demonstrated a role for COPI in ER-to-Golgi traffic (see Nickel and Wieland 1998). More recently, a role for COPI in Golgi-to-ER retrograde transport has been proposed, based on yeast genetic data (Letourneur et al. 1994; Pelham 1994). Whether COPI that is bound to pre-Golgi intermediates and Golgi membranes is responsible for retrieval of proteins back to the ER or is involved in forward trafficking is, therefore, a subject of much debate. The answer to this question is fundamental for understanding the role of the ARF1/COPI complex in the formation and functioning of the secretory trafficking system (see below).

The third task of a functional secretory apparatus, fusion, requires the involvement of cytosolic and membrane proteins to regulate specific targeting and membrane fusion events between transport intermediates. These include a large family of small ras-related GTPases (Rab proteins), and a family of membrane proteins that play a central role in compartment-specific docking (the v- and t-SNARES; see Rothman and Sollner 1997). Found on transport intermediates, the Rab proteins were originally thought to specify prefusion docking events. It now appears that the function of the Rab family of proteins is more complex. Recent evidence points to their role in regulating the directional movement of transport intermediates along microtubules (Echard et al. 1998), as well as for controlling the rate of fusion between specific transport intermediates (Rybin et al. 1996; Lupashin and Waters 1997). The latter function is likely accomplished by facilitating the interaction between members of the docking/fusion machinery, the t-SNARES and v-SNARES (Rothman 1994), an interaction that is normally inhibited by another family of proteins, the Sec1 proteins (Pevsner 1996; Lupashin and Waters 1997). Together with the other members of the docking/fusion machinery (NSF/SNAP; see Rothman 1994), these molecular components regulate fusion and assembly of post-ER membranes and establish trafficking patterns.

Towards a mechanistic model of secretory traffic: role of ARF1/COPI in organelle maintenance and membrane transport

ARF1/COPI complexes have traditionally been thought to function in the formation of coated vesicles for membrane transport (Rothman and Wieland 1996; Schekman and Orci 1996). Experimental results over the last several years addressing the regulation of these molecules suggest membrane association of ARF1/COPI is likely to have a broader role (Klausner et al. 1992; Dascher and Balch 1994; Zhang et al. 1994; Peters et al. 1995; Gaynor et al. 1998). In particular, ARF1/COPI association with membranes appears to be crucial for the formation of the Golgi complex and its proper functioning in secretory traffic (see model in Fig. 4).

A fundamental feature of secretory traffic is the ability to segregate surface-destined membrane components from ER/Golgi resident components. This process involves sorting/partitioning of molecules into highly organized membrane domains and is required both for the initial formation of pre-Golgi structures and their maturation into Golgi elements. The activity of Sar1p/COPII appears to be crucial for initiating the organization of these structures and leads to concentration of selective cargo at ER exit sites (Balch et al. 1994; Barlowe et al. 1994; Kuehn et al. 1998). Because it acts only at this location, however, Sar1p/COPII is insufficient to complete the job of membrane transport and organellogenesis. For

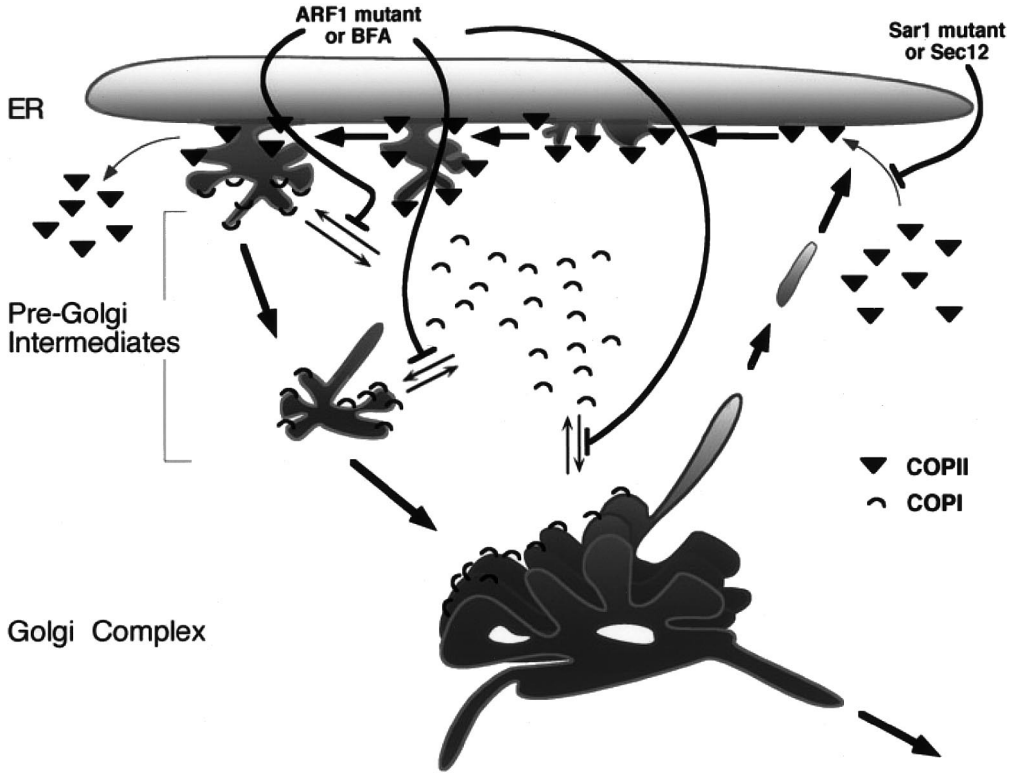

Fig. 4 Model depicting concentration/sorting processes involved in Golgi biogenesis and membrane trafficking and the potential roles of SAR1/COPII and ARF1/COPI in these processes. Export of proteins out of the ER is initiated by assembly of Sar1/COPII onto membranes, which leads to concentration of protein and lipid at the site of binding. This, in turn, causes ER membrane at this site to transform into budding tubules/vesicles. Binding of ARF1/COPI to these regions leads to their further differentiation, including the recruitment and concentration of other membrane components. Soon clusters of tubule/vesicles arises (i.e. pre-Golgi intermediates). If such structures interact with microtubules, they can detach from the ER system and move toward the center of the cell where they fuse to form the *cis* face of the Golgi complex. Molecules that do not partition efficiently into the ARF1/COPI sorting domains of pre-Golgi intermediates recycle back to the ER. Expression of the dominant-negative Sar1 mutant (T39N) or, in yeast, the sec12 mutant (sec12–1_{ts}) blocks the assembly of COPII onto ER membranes and export out of the ER, as indicated. Expression of the dominant-negative ARF1 mutant (T31N) or treatment with BFA blocks COPI assembly and subsequent export of cargo from ER to Golgi, as shown. These blocks also result in redistribution of Golgi membrane proteins back to the ER (Lippincott-Schwartz et al. 1990 and unpublished results, as predicted if Golgi-resident proteins cycle continuously within the ER/Golgi system

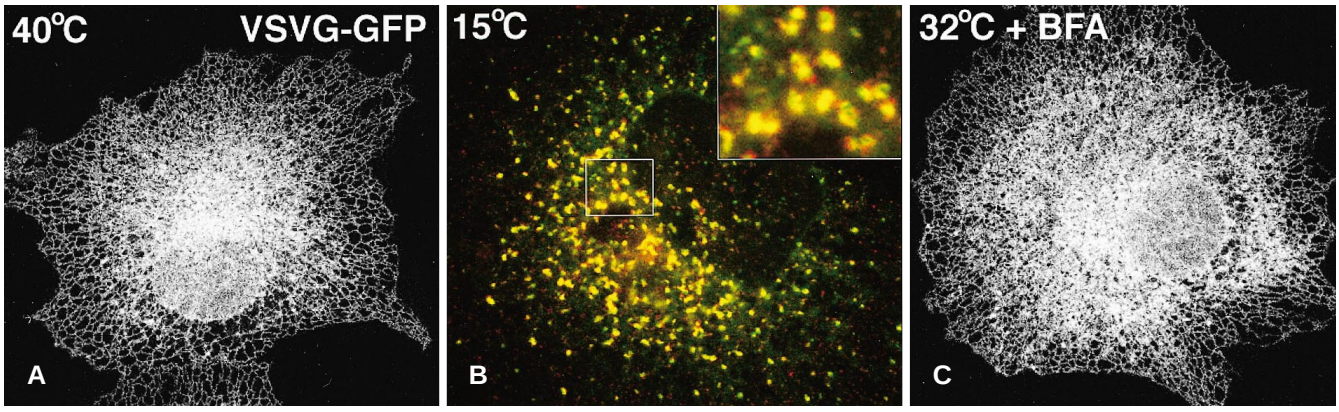

Fig. 5A–C Cargo proteins such as VSVG fail to cluster at ER exit sites or concentrate in pre-Golgi intermediates in cells where COPI is prevented from binding to membranes as a result of BFA treatment. A temperature-sensitive variant of VSVG tagged with green fluorescent protein (VSVG-GFP) (Presley et al. 1997) misfolds and is retained in the ER at 40°C (*left* panel **A**) but is capable of moving into pre-Golgi intermediates/Golgi elements upon shift to 32°C or 15°C (*middle* panel **B**). Pre-Golgi intermediates that accumulate VSVG-GFP at 15°C (see green GFP staining) are enriched in COPI (see red antibody staining) with significant overlap in the two markers observed (see merged yellow signal). If cells are shifted from 40°C to 32°C in the presence of BFA, where COPI is prevented from binding to membranes, very little or no accumulation of VSVG-GFP is observed in pre-Golgi intermediates or Golgi elements (*right* panel **C**). These data suggest that membrane binding of ARF1/COPI is critical for the recruitment of cargo proteins into ER exit sites

this, surface-destined membrane components must be selectively retained within forward moving membrane domains as transport machinery necessary for ER export and Golgi enzymes recycle back to the ER system. Growing evidence indicates that ARF1/COPI helps to perform this job.

COPI binding to membranes requires activation of ARF1 GTPase, analogous to the dependence of Sar1-GTP for COPII binding. Sequential binding of Sar1/COPII followed by ARF1/COPI at ER exit sites (Rowe et al. 1996) indicates that association of ARF1/COPI to membranes arises after these membranes have been first differentiated by the activity of Sar1/COPII. ARF1/COPI binding appears to play a critical role in the further differentiation of these sites (see model in Fig. 4). Not only are additional proteins recruited to ER exit sites upon ARF1/COPI binding but, as pre-Golgi intermediates mature into Golgi elements, retention of the concentrated assembly of proteins within these structures is critically dependent on ARF1/COPI binding. This interpretation is based on in vivo studies which have shown that cargo proteins such as VSVG, as well as Golgi enzymes, fail to efficiently cluster into ER exit sites or concentrate in pre-Golgi intermediates in cells where COPI is prevented from binding to membranes (e.g., in cells expressing ARF dominant-negative mutants and in cells treated with BFA; see Fig. 5) even though Sar1/COPII still binds (Lippincott-Schwartz et al. 1990; Dascher and Balch 1994; Peters et al. 1995). Thus, in the absence of ARF1/COPI activity at ER exit sites, the action of Sar1p/COPII is insufficient to sort into pre-Golgi intermediates the diverse array of molecules that are required to build and maintain the secretory apparatus. Such studies have also revealed that ARF1/COPI activity is required for maintaining these proteins in pre-Golgi and Golgi elements after they have formed. In the absence of ARF1/COPI binding to membranes, proteins within pre-Golgi and Golgi membranes redistribute back into the ER via retrograde tubule carriers (see Fig. 6) (Lippincott-Schwartz et al. 1990; Sciaky et al. 1997).

A role of ARF1/COPI in forward secretory traffic has been implicated in many studies. In vitro studies reconstituting ER-to-Golgi transport have shown that inhibitory mutants of ARF1 block ER-to-Golgi traffic and prevent ARF1/COPI assembly onto pre-Golgi intermediates that have lost the Sar1/COPII coat (Dascher and Balch 1994; Rowe et al. 1996). In addition, COPI-coated, pre-Golgi structures have been observed to translocate through the cytoplasm to the Golgi complex in living cells (Presley et al. 1997; Scales et al. 1997), and microinjection of antibodies to COPI subunits disrupts this forward membrane trafficking (Pepperkok et al. 1993; Peter et al.1993). Temperature-sensitive mutants of COPI subunits are defective in transport in the early secretory pathway in mammalian cells (Guo et al. 1994) and in yeast (Schekman and Orci 1996). Finally, analysis of the effects of Arf1 deletion in yeast has revealed inhibition of forward secretory traffic with minimal effect on retrieval pathways (Stearns et al. 1990; Gaynor et al. 1998).

At least two properties of ARF1/COPI and its interaction with membranes make this protein complex an attractive candidate for facilitating the temporal and spatial processes involved in pre-Golgi and Golgi maturation and trafficking. First, given that ARF activation is required for COPI binding and that ARF has low intrinsic GTPase activity, ARF regulators [for example, GTP exchange factors (GEFs) to exchange GTP for GDP, and GTPase activating proteins (GAPs) to convert ARF-GTP to its inactive ARF-GDP form] are likely to be required to modulate the repetitive cycles of activation of ARF on membranes. These ARF regulators, therefore, could provide enormous flexibility in controlling the temporal and spatial specificity of ARF1/COPI binding to membranes. Second, in addition to promoting COPI binding to membranes, ARF has been shown to be an activator of phospholipase D (Brown et al. 1993; Cockcroft et al. 1994), which catalyzes the hydrolysis of phosphotidyl choline to form phosphatidic acid (PA). The production of PA may be involved in creating the COPI binding site by altering membrane curvature (Ktistakis et al. 1996). Furthermore, PA can subsequently be converted to other biologically active lipids that could modulate the behavior of pre-Golgi and Golgi membrane lipids. ARFs have also been suggested to alter PIP and PIP_2 levels in cells (Fensome et al. 1996) which, in turn, could affect the activities of ARF-GEFs and GAPs (Randazzo and Kahn 1994; Liscovitch and Cantley 1995). Thus, ARF's actions can modulate membrane composition (and possibly morphology) in addition to promoting COPI assembly to membranes.

The above findings strongly suggest that membrane association of ARF1/COPI is necessary for maintenance of the compartmental organization of pre-Golgi and Golgi membranes, but how they accomplish this is unclear. Recent studies using yeast genetics have suggested that COPI binding to pre-Golgi and Golgi membranes is involved in mediating retrograde traffic through the production of COPI-coated vesicles. This is based on the finding that COPI subunits can bind to dilysine motifs (Cosson and Letourneur 1994) which are found on the C-terminus of several proteins believed to function as ER retrieval sequences (Nilsson et al. 1989). In a screen designed to detect mutants unable to retain/recycle dilysine-tagged reporter molecules, but still carry on forward anterograde traffic, mutant COPI subunits were identified (Letourneur et al. 1994). These observations led to the proposal that COPI is required for retrograde traffic, with any effects on anterograde traffic explained by the failure to recycle critical components back to the ER (Pelham 1994; Gaynor and Emr 1997). Although COPI, through this screen, is genetically linked to retention/retrieval of these dilysine-containing proteins, the mechanism whereby COPI serves this function is not clear. Retention versus retrieval of proteins in a membrane cycling system is difficult to distinguish, especially when based primarily on genetic data. Dilysine motifs in mammalian cells are present on many ER resident proteins which never leave the ER (Tisdale and Jackson 1996), as

well as on a number of Golgi resident proteins (Schroder et al. 1995; Stamnes et al. 1995; Sohn et al. 1996; Dominguez et al. 1998). Since COPI is associated with Golgi and not ER membranes, how dilysine-containing proteins interact with COPI in different membrane compartments is unclear.

The most powerful argument against the idea that COPI mediates retrograde transport through the formation of COPI-coated vesicles comes from analysis of the role of ARF1 in COPI binding. All evidence to date indicates that COPI binding to membrane requires activation of ARF. If COPI mediates retrograde transport, a prediction is that ARF inhibition should block retrograde transport. In fact, ARF inhibition enhances retrograde traffic and leads to a block in protein export out of the ER. As described below, these observations suggest membrane association of ARF1/COPI has an alternative role in membrane trafficking than the production of COPI retrograde vesicles.

An unambiguous method for defining the role of a GTP-binding protein is to investigate the effect of expressing the dominant-negative form of the protein. These mutants have an altered critical threonine residue that renders them defective for GTP binding. More importantly, however, their expression sequesters the GEF and thereby blocks the activation of the endogenous GTP-binding protein (Boguski and McCormick 1993). The observed loss of function is usually the site where the activation of the GTP-binding protein is required. For ARF1, the dominant-negative T31N mutant not only prevents COPI binding to membranes but blocks all protein export out of the ER. It also results in Golgi membrane redistribution into the ER since Golgi proteins are no longer actively retained in Golgi membranes and recycle back to the ER (Dascher and Balch 1994; Peters et al. 1995). These observations indicate, therefore, that ARF1/COPI is not necessary for retrograde transport of membrane proteins back to the ER. The data instead suggest that ARF1/COPI association with membranes is important for sorting/retention of molecules within pre-Golgi and Golgi structures. This activity could serve to limit the type and extent of retrograde traffic back to the ER, and in so doing allow secretory organelles to differentiate and mature.

Similar observations have been made in mammalian and yeast cells treated with BFA (Lippincott-Schwartz et al. 1990; Graham et al. 1993; Rambourg et al. 1995), which acts by inhibiting nucleotide exchange onto ARF catalyzed by Golgi-associated GEFs (Donaldson et al. 1992b; Helms and Rothman 1992). Evidence that BFA's action within cells is via its inhibition of ARF1 activation is supported by the finding that a BFA-resistance factor isolated from mutant BFA-resistant CHO cells is an ARF-GEF (Melançon et al. 1997) and because BFA-inhibitable GEFs have now been cloned from both mammalian cells and yeast (Morinaga et al. 1996; Peyroche et al. 1996). Thus, BFA treatment provides a useful tool for investigating the acute effects of ARF inactivation and COPI dissociation from membranes. Within 30 s after adding BFA to mammalian cells, COPI is no longer associated with Golgi membranes due to the failure of ARF to be activated (Donaldson et al. 1990). This results in an immediate block in protein export out of the ER in vivo (Lippincott-Schwartz et al. 1990). Subsequently, Golgi membranes start to become disorganized and incapable of retaining protein and lipid, with Golgi protein and lipid entering retrograde tubule processes that move outward into the cytoplasm (Fig. 6). In untreated cells, the protein content in such retrograde tubules is regulated and they detach from the Golgi complex before moving out to the cell periphery (Sciaky et al. 1997). In BFA-treated cells, however, the protein content in retrograde tubules is less specific and the tubules do not detach from the Golgi body. This leads to rapid emptying of all Golgi contents into the ER when one or more of the tubules fuses with the ER. The irreversible absorption of Golgi lipid and protein into the ER observed under these conditions suggests the ER provides a lower free energy environment for protein and lipid than the Golgi complex. A possible source for the free energy difference between ER and Golgi membranes could be from Sar1p/COPII and ARF1/COPI activities, which sort and concentrate membrane proteins into pre-Golgi intermediates, thereby 'pumping up' Golgi-destined material in energy (Sciaky et al. 1997).

In contrast to the effects of ARF inhibition, persistent activation of ARF1 with GTPγ S or with the constitutively active mutant, ARF1/Q71L, results in irreversible coating of membranes with COPI, massive non-specific budding of Golgi membranes, resistance to the effects of BFA, and a block in both anterograde and retrograde traffic (Melançon et al. 1987; Dascher and Balch1994; Teal et al. 1994; Zhang et al. 1994). These observations support the previous proposal that ARF1 and COPI have a role in maintaining the structure of the Golgi complex and in regulating the extent of retrograde traffic (Klausner et al. 1992; Lippincott-Schwartz 1993). Since then, evidence for constitutive recycling of Golgi-localized proteins back to the ER using an assay based on the capacity of the ER to retain misfolded proteins has suggested that retrograde transport to the ER may be an inherent feature of Golgi resident proteins that does not require specific retrieval signals (Cole et al. 1998). Other work has shown that the vehicles for retrograde transport are not necessarily small vesicles and can be tubules. In particular, time-lapse images of green fluorescent protein-tagged KDEL receptor (KDELR), which constitutively recycles between the ER and Golgi complex, revealed KDELR-enriched tubules (not vesicles) moving out of Golgi elements to the cell periphery along microtubule tracks (see Fig. 6) (Sciaky et al. 1997).

Given the above results, the idea that ARF1/COPI activity is directed solely to the production of COPI-coated vesicles for retrograde traffic is not very compelling. Notably, it is the inhibitors of ARF1 (i.e., expression of T31N ARF and BFA treatment) that block COPI association to membrane and enhance retrograde traffic. This implies that ARF/COPI activity is not required for Gol-

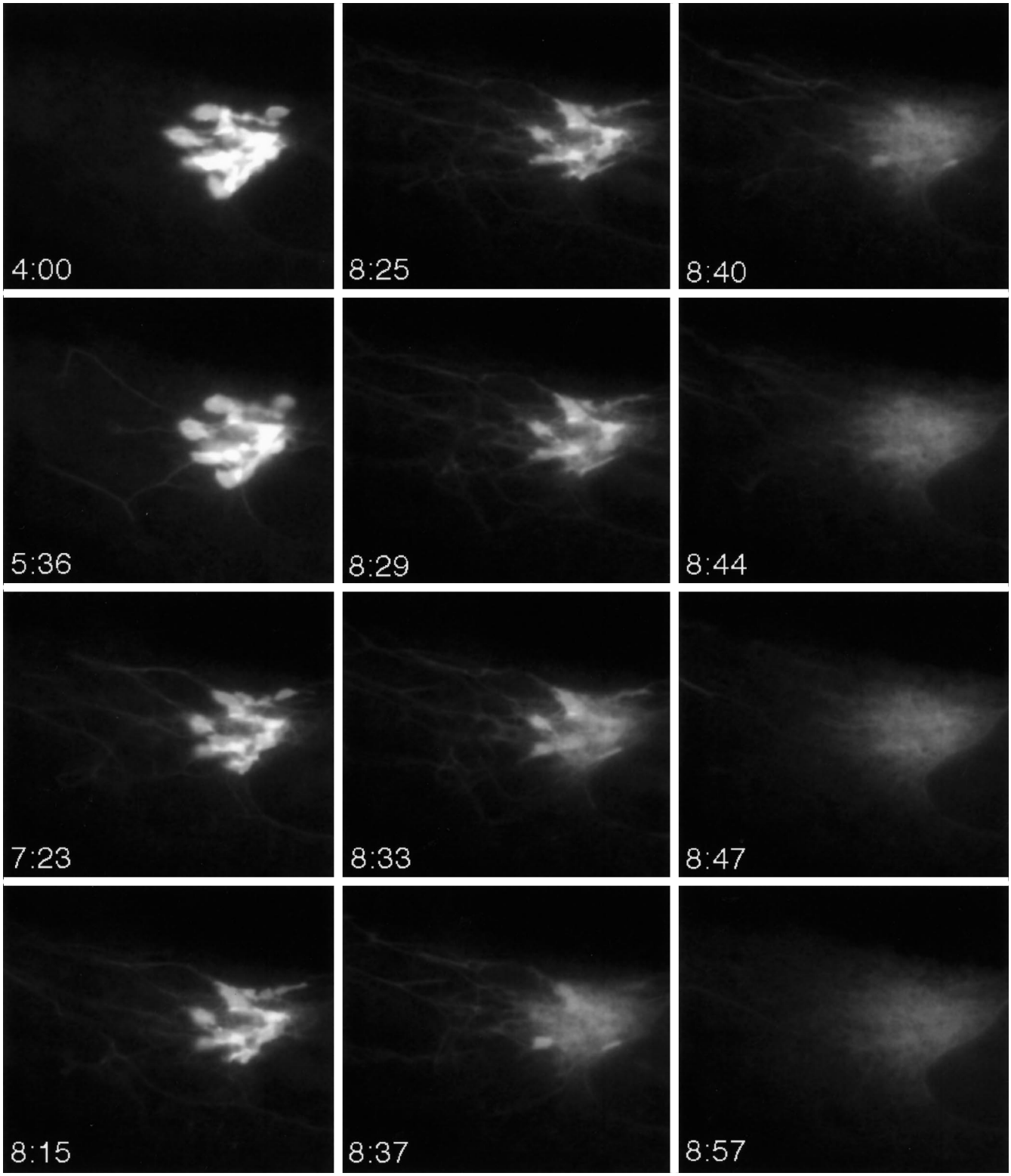

Fig. 6 Golgi tubulation and redistribution into the ER in cells treated with BFA. HeLa cells expressing the Golgi enzyme galactosyltransferase tagged with GFP (GalTase-GFP) were visualized after addition of BFA. Within 4 min (*4:00*), small tubules emerging from the Golgi began to proliferate. Over the next 3 min they grew to form a tubular network still attached to the Golgi body. When one or more of the tubules fused with the ER, all of the Golgi marker rapidly emptied into the ER in a period of about 14 s (beginning at 8:33). *Bar* 5 μm. (Reprinted with permission of Sciaky et al. 1997). See Quicktime movie of this sequence at http://dir.nichd.nih.gov/CBMB/pb4labob.htm. The source of the free-energy difference between ER and Golgi membranes that leads to unidirectional transport into the ER when these membranes fuse upon BFA treatment is not known. One possibility is that the work required to 'pump up' Golgi-destined material in energy is supplied by the peripheral coat proteins (Sar1/COPII and ARF1/COPI), which concentrate membrane proteins and cargo exported from the ER

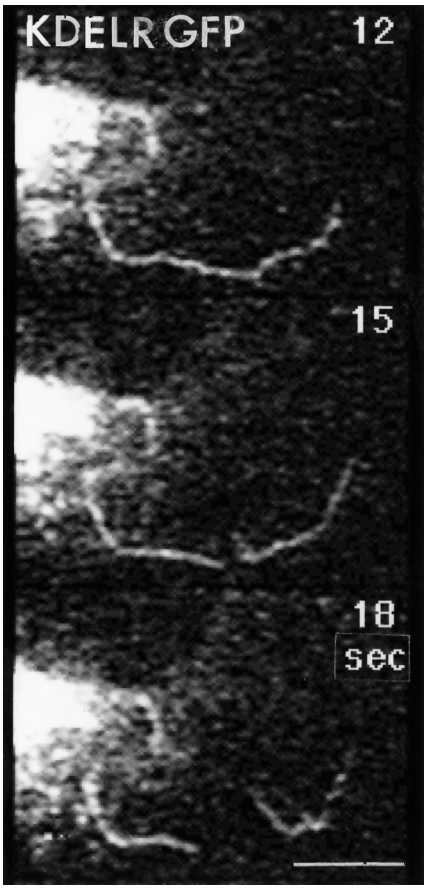

Fig. 7 Tubule activity of Golgi membranes observed with KDEL receptor (KDELR) tagged with GFP (*KDELR-GFP*). Images were collected at 3-s time intervals on a confocal microscope. Golgi tubules containing KDELR-GFP were observed to extend rapidly, break off, and continue to move out to the cell periphery at speeds of 0.6 μm/s. Tubule extension and translocation required microtubules. Because KDELR recycles to the ER, Golgi tubules carrying these proteins to the cell periphery are likely to represent retrograde transport intermediates. *Bar* 3 μm

gi-to-ER traffic. Recent observations made with yeast Arf1 deletion strains are consistent with this thinking since such strains showed marked inhibition of forward traffic and minimal effects on retrieval functions (Gaynor et al. 1998). It also fits with the recent observation that overexpression of a GAP for ARF1 leads to redistribution of Golgi membranes into the ER, which can be rescued by overexpression of ARF1 (Aoe et al. 1997). Interestingly, ARF-GAP has been found to associate with KDELR in cells overexpressing this receptor (Aoe et al. 1997). This association could provide an explanation for an earlier observation that KDELR overexpression leads to Golgi protein redistribution into the ER (Hsu et al. 1992). If ARF-GAP is recruited onto membranes as a result of KDELR overexpression, hydrolysis of ARF1-GTP by ARF-GAP could result in localized COPI dissociation from membrane, resulting in tubule formation that could carry membrane proteins localized at this site back to the ER (Fig. 7). Under such a model, the ARF1/COPI complex might help recruit proteins destined for retrieval (including dilysine-containing proteins) to regions of the Golgi complex where, after hydrolysis of GTP-bound ARF, retrograde tubule carriers arise.

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